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37 C.F.R. §1.8

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3/15/04
Date

Steven L. Highlander

**PATENT**

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

*In re* Application of:
Sujata KALE and
Michael W. LONG

Serial No.: 09/753,043

Filed: December 27, 2000

For: PROCESS FOR EX VIVO FORMATION OF MAMMALIAN BONE AND USES THEREOF

Group Art Unit: 1636

Examiner: Jean C. Witz

Atty. Dkt. No.: UMIC:048US/SLH

**SECOND DECLARATION OF MICHAEL LONG UNDER 37 C.F.R. § 1.132**

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-01450

Dear Sir:

1. I am a citizen of the United States of America, residing at 570 High St., Northville MI 48167.

2. I am the Michael W. Long named as an inventor on the above-captioned patent application. I have been conducting research in the area of bone formation and repair for 18 years. A copy of my *curriculum vitae* has been submitted with my previous declaration.

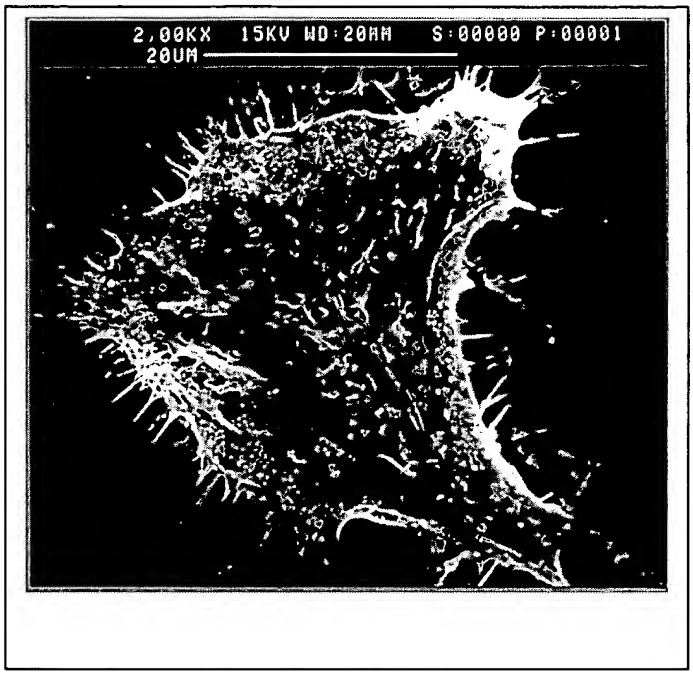
3. Based on a review of the Office Action mailed on April 4, 2003, it is my understanding that the examiner for above-captioned application continues to suggest that the bone "nodules" or "globules" described in U.S. Patent 6,152,964 are the same as the bone spheroids of the instant application. Again, I believe the examiner to be incorrect in this supposition.

4. As stated in my previous declaration, there is a considerable difference in the size of bone cell spheroids and what the '964 nodules. FIGS. 1 and 4 in the '964 patent are SEM photos (10 μm bar in white at the bottom). The material being described in those tissue is, by definition, acellular, since nothing even close to 10 μm in diameter is shown. The bone cell spheroids we develop as part of this invention consist of 10,000 to 100,000+ cells. They are thus much larger in size. Likewise the bone synthesized by the cells of the spheroid is larger than the structures apparent in FIGS. 1 and 4 of the '964 patent.

5. The osteoblast is a bone-forming cell. It is large (25 to 50 μm in diameter), often ellipsoid, and contains a round or ovoid nucleus with one or more nucleoli. The cytoplasm is abundant and stains blue-gray. A prominent clear zone (Golgi), a small distance away from the nucleus, is usually evident (source: www.courses.ahc.umn.edu/medical-school/ LaMP/5104/atlas/glossary.htm). "Osteoblasts ... they vary in size and shape, most being 20-30 micrometers in diameter" (source: medic.med.uth.tmc.edu/edprog/ histolog/blood/hist-08.htm - 10k –): A photograph of a typical osteoblast is shown in FIG. A (source: www.eastman.ucl.ac.uk/~jknowles /lectures /EDI%20 Research%20Seminar%20180401%20

Vehid%20Salih.ppt). FIG. B shows photomicrographs of cultured osteoblasts (source: Moursi *et al.*, *J. Cell Sci.* 109, 1369-1380 (1996), indicating that their size is in excess of 40 microns in diameter.

**FIG. A** - 20+ Micron Osteoblast SEM



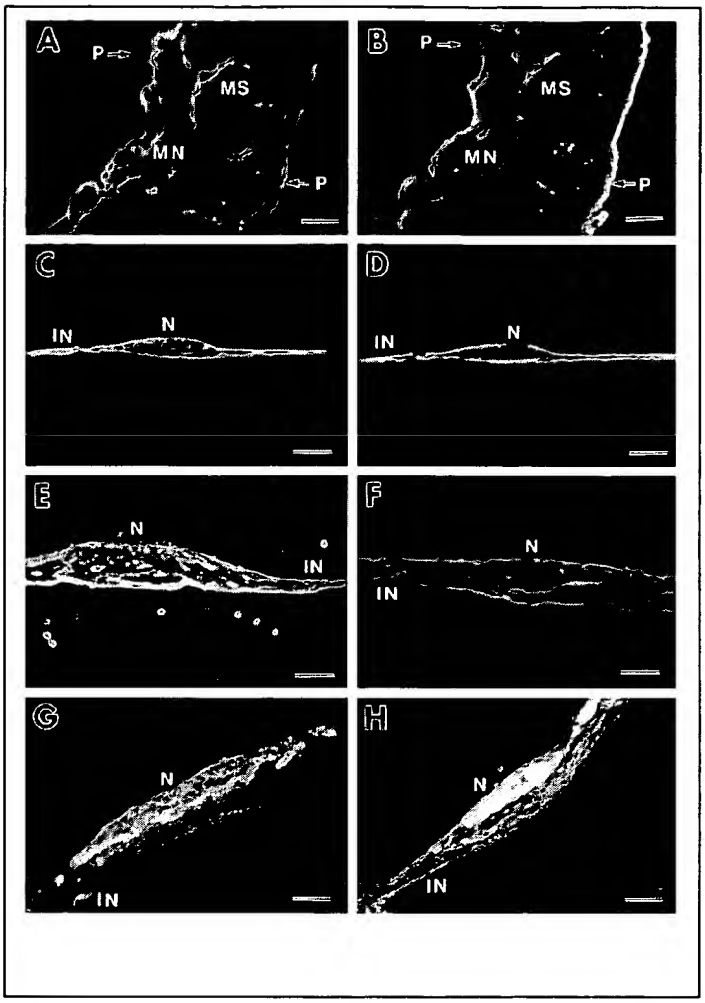



**FIG. B** - Extracellular matrix components have distinct distribution patterns in fetal rat calvaria and differentiated osteoblast cultures. Cryostat cross-sections of fetal 21 day-old calvaria were incubated with antibodies against the α5 integrin subunit (A) and FN (B). α5 staining was strongest in the periosteal surface adjacent to bone (P). FN staining was strongest in the periosteal surface adjacent to bone, with little localization in the mineralized tissue (MN). **8 day osteoblast cultures, corresponding to nodule initiation,** were incubated with antibodies against the α5 integrin subunit (C) and FN (D). **14-day osteoblast cultures** were incubated with antibodies against α5 (E), FN (F), osteopontin (G) and osteocalcin (H). In cultured osteoblasts α5 staining was most intense in the internodular (IN) regions and the periphery of the nodules (N). Some cells within the nodule (N) also stained for α5. FN was detected in the internodular region (IN) and around the periphery of the nodule (N), but not within the nodule itself. Staining for osteopontin and osteocalcin was confined largely to the core of the nodule (N). All samples were incubated with the appropriate secondary antibodies conjugated to rhodamine. MS, marrow space. **Bars, 40 μm.**

6. Thus, based on this size information, it is clear that the materials examined in the '964 patent cannot be cellular, much less a collection of 3000 to 100,000 cells.

7. I hereby declare that all statements made herein of my knowledge are true and that all statements made herein on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both, under § 1001 of Title 18 of the U.S. Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

_____ _____

Date Michael W. Long, Ph.D.